PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	11 MAY 2005
Applicant's or agent's file reference 14375-003WO1	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/31933	29 September 2004 (29.09.2004)	29 September 2003 (29.09.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12N 5/02, 5/06; A61B 17/42, 17/435, 17/46 and US Cl.: 435/325, 374; 424/93.1, 93.7; 600/33, 34

Applicant

EMBRYONICS, INC

1. This opinion contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Michael Wityshyn Telephone No. 571-272-1600

Form PCT/ISA/237 (cover sheet) (January 2004)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/31933

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing

      ☐ contained in international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 52-56,72-99 and 106-126

because:

☐ the said international application, or the said claim Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 52-56,72-99 and 106-126 are so unclear that no meaningful opinion could be formed *(specify)*:

Claim 52 is so unclear that no meaningful opinion can be formed. Claims 53-56, 72-99 and 106-126 are improper mulitple dependent claims. Note when a claim reads "the method of any of the above claims" it refers to all previous claims, thus if any preceeding claim is a multiple dependent, either proper or improper, the claim in question is considered an improper mulitple dependent claim. Also, any claim depending from an improper mulitple dependent claim is also improperly mulitply dependent. PCT Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-51, 57-71 and 100-105	YES
	Claims NONE	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-51, 57-71 and 100-105	NO
Industrial applicability (IA)	Claims 1-51, 57-71 and 100-105	YES
	Claims NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

International application No.
PCT/US04/31933

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:
Claims 1-51, 57-71 and 100-105 lack an inventive step under PCT Article 33(3) as being obvious over Wells et al (Fertility and Sterility, 2002) in view of Toner et al (US 2002/0045156 A1), further in view of Gook et al (Human Reprod, 1993).

Wells et al teach a method of analyzing first polar bodies from oocytes in order to diagnose genetic disorders related to aneuploidy before implantation of the fertilized oocyte. Wells et al teach that more than half of all human embryos to be used in IVF contain chromosomal imbalances, resulting in spontaneous abortion or genetic disorders. In order to increase the effectiveness of IVF treatments resulting in normal, healthy offspring Wells et al teach a method of testing the first polar body of harvested oocytes by whole genome amplification followed by comparative genomic hybridization, in order to determine if the corresponding oocyte is aneuploid. Wells et al fertilize all oocytes prior to testing the corresponding polar bodies, and only implanting those embryos that came from normal haploid oocytes, discarding the aneuploid embryos; however, it would have been obvious to test the polar bodies before fertilization in order to prevent destruction of embryos and unnecessary costs associated with the excess fertilizations.

Though Wells et al performs the testing of the polar bodies on the same day as the fertilization of the oocytes takes place, it would have been obvious to one of ordinary skill in the art to test the polar bodies associated with the oocytes, store only the desired oocytes for fertilization treatments at a later date, and then revive the stored oocytes from storage at a desired time point; wherein data and labels are used to ensure proper correlation between the tested polar bodies and the stored oocytes. Because only oocytes suitable for use in IVF are selected for storage, Wells et al's method also determines the suitability of oocytes for storage. One would have been motivated to store the desired oocytes for use at a later date in the case of females undergoing chemotherapy treatments that render them infertile, harvesting and storage of viable oocytes prior to treatment can then be reused after treatment. It would be desirable to immediately test the polar bodies associated with the oocytes and then store only the desired, normal oocytes, discarding the aneuploid, or otherwise flawed oocytes, in order to reduce costs associated with storage.

Appropriate storage methods are disclosed by Toner et al. Toner et al teach a method for cryopreservation of oocytes via microinjection of a cryoprotectant, comprising microinjecting into the cytoplasm of an oocyte a protective agent that is substantially non-permeating with respect to mammalian cell membranes and maintains the viability of the cell to that it can be stored in a temporarily dormant state and restored to an active state; subjecting the microinjected cell to conditions to cause it to enter a dormant state; store the cell; and then subsequently restoring the cell to an active state when desired. The preservation agent is to be used in low levels less than 0.4M; it may consist of only a sugar, such as sucrose (glass transition temp -32°C), trehalose (glass transition temp -29.5°C), or lactose (glass transition temp -28°C), or such a sugar in combination with a conventional cryoprotectant. The culture medium is to have an osmolarity of at least 300 mOsm. The cytoplasmic concentration of the sugar is less than 0.01M after microinjection. An additional extracellular protective agent that is substantially non-permeating to the cells, such as the sucrose/propanediol solution described by Gook et al, may also be used. The extracellular concentration of the sugar is also to be less than 0.01M in the medium containing the cell. Alternatively, the protective agent may comprise a glycolipid or a glycoprotein that has a molecular weight of at least 120 daltons. Once the oocyte cells are prepared for storage with the proper protective agents, Toner et al teach the oocytes are to be frozen or dried, via plunge freezing, vacuum drying, air drying, or freeze drying. Oocytes can be cooled at a rate of 0.1°C/min to a final temperature that can be as low as -60°C. Dried oocytes can be stored appropriately at room temperature. Toner et al teach the oocytes can be returned to an active state by rehydration or thawing. The oocytes can then be returned to the appropriate culture medium, that has an osmolarity of greater than 300 mOsm, preferably greater than 380 mOsm. Upon restoration to a viable state the oocytes can be used in fertility treatments including

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

invitro fertilization.

One would have expected success storing the oocytes, selected by the method of Wells et al, by the method of Toner et al because Toner et al teach oocytes can successfully be subjected to cryogenic or dried storage by means of their method, and then returned to active states to produce viable embryos for use in reproductive therapy treatments.

Claims 1-51 and 57-71 lack an inventive step under PCT Article 33(3) as being obvious over Ebner et al (Fertility and Sterility, 1999) in view of Toner et al (US 2002/0098470 A1), further in view of Gook et al (Human Reprod, 1993).

Ebner et al teach a method of determining the suitability of oocytes for use in ICSI by examining the morphology of the first polar body associated with the oocytes. Oocytes associated with polar bodies with normal morphologies are selected for IVF treatments; oocytes associated with abnormal morphologies are discarded.

Though Ebner et al performs the testing of the polar bodies on the same day as the fertilization of the oocytes takes place, it would have been obvious to one of ordinary skill in the art to test the polar bodies associated with the oocytes, store only the desired oocytes for fertilization treatments at a later date, and then revive the stored oocytes from storage at a desired time point; wherein data and labels are used to ensure proper correlation between the tested polar bodies and the stored oocytes. Because only oocytes suitable for use in IVF are selected for storage, Ebner et al's method also determines the suitability of oocytes for storage. One would have been motivated to store the desired oocytes for use at a later date in the case of females undergoing chemotherapy treatments that render them infertile, harvesting and storage of viable oocytes prior to treatment can then be reused after treatment. It would be desirable to immediately test the polar bodies associated with the oocytes and then store only the desired, normal oocytes, discarding the aneuploid, or otherwise flawed oocytes, in order to reduce costs associated with storage.

Appropriate storage methods are disclosed by Toner et al. Toner et al teach a method for cryopreservation of oocytes via microinjection of a cryoprotectant, comprising microinjecting into the cytoplasm of an oocyte a protective agent that is substantially non-permeating with respect to mammalian cell membranes and maintains the viability of the cell to that it can be stored in a temporarily dormant state and restored to an active state; subjecting the microinjected cell to conditions to cause it to enter a dormant state; store the cell; and then subsequently restoring the cell to an active state when desired. The preservation agent is to be used in low levels less than 0.4M; it may consist of only a sugar, such as sucrose (glass transition temp -32°C), trehalose (glass transition temp -29.5°C), or lactose (glass transition temp -28°C), or such a sugar in combination with a conventional cryoprotectant. The culture medium is to have an osmolarity of at least 300 mOsm. The cytoplasmic concentration of the sugar is less than 0.01M after microinjection. An additional extracellular protective agent that is substantially non-permeating to the cells, such as the sucrose/propanediol solution described by Gook et al, may also be used. The extracellular concentration of the sugar is also to be less than 0.01M in the medium containing the cell. Alternatively, the protective agent may comprise a glycolipid or a glycoprotein that has a molecular weight of at least 120 daltons. Once the oocyte cells are prepared for storage with the proper protective agents, Toner et al teach the oocytes are to be frozen or dried, via plunge freezing, vacuum drying, air drying, or freeze drying. Oocytes can be cooled at a rate of 0.1°C/min to a final temperature that can be as low as -60°C. Dried oocytes can be stored appropriately at room temperature. Toner et al teach the oocytes can be returned to an active state by rehydration or thawing. The oocytes can then be returned to the appropriate culture medium, that has an osmolarity of greater than 300 mOsm, preferably greater than 380 mOsm. Upon restoration to a viable state the oocytes can be used in fertility treatments including invitro fertilization.

One would have expected success storing the oocytes, selected by the method of Ebner et al, by the method of Toner et al because Toner et al teach oocytes can successfully be subjected to cryogenic or dried storage by means of their method, and then returned to active states to produce viable embryos for use in reproductive therapy treatments.

Claims 1-51, 57-71 and 100-105 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.